

AD _____
(Leave blank)

Award Number: W81XH-11-2-0033

TITLE: Advanced Cancer Genomics Institute: Genetic Signatures and
Therapeutic Targets in Cancer Progression

PRINCIPAL INVESTIGATOR: Irwin H. Gelman, Ph.D.

CONTRACTING ORGANIZATION: Health Research Inc., Roswell Park Cancer
Institute Division, Buffalo, NY 14263

REPORT DATE: February 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 02/12/14		2. REPORT TYPE Annual		3. DATES COVERED 31 Sept 2013 - 31 Dec 2013	
4. TITLE AND SUBTITLE ADVANCED CANCER GENOMICS INSTITUTE: GENETIC SIGNATURES AND THERAPEUTIC TARGETS IN CANCER PROGRESSION				5a. CONTRACT NUMBER W81XWH-11-2-0033	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Irwin H. Gelman, Ph.D. E-Mail: irwin.gelman@roswellpark.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Roswell Park Cancer Institute Elm and Carlton Streets Buffalo, NY 14263				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Our aim is to develop multiple next-gen-based genomics analysis capabilities to be able to identify cancer-related signatures that predict progression to malignancy and that identify novel therapeutic pathways and targets. To this end, we are purchasing for the Advanced Cancer Genomics Institute within our Genomics Core resource Lab multiple next-gen instruments, including associated hardware and software platforms. Following the performance of quality-assurance testing, RPCI researchers will perform whole genome sequencing/re-sequencing, exome-seq, transcriptome-seq, miRNA-seq, ChIP-seq, microbiome-seq, virome-seq and copy number variation/loss of heterozygosity (CNV/LOH) analyses including the associated bioinformatics analyses. Based on the concentration of expertise at RPCI in prostate cancer pre-clinical and clinical models, most of the proposed work has been adapted to address genomic signatures of androgen receptor-driven progression to castration-recurrent prostate cancer (CR-CaP). The capabilities of this Core will foster our goal of developing practical approaches to personalized cancer medicine. The long-term aim is to develop full-operational next-gen expertise to facilitate the collaboration of RPCI researchers with other members of the National Functional Genomics Consortium.					
15. SUBJECT TERMS Next-generation sequencing technologies, exome-seq, transcriptome-seq, miRNA-seq, ChIP-seq, microbiome-seq, virome-seq, genomic signatures, personalized cancer medicine					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	13	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Executive Summary.....	4
Grant Chart.....	5
Introduction.....	6
Body.....	6
Key Research Accomplishments.....	6
Reportable Outcomes.....	11
Conclusion.....	12
Quarterly Report Summary.....	14
References.....	N/A
Appendices.....	N/A

EXECUTIVE SUMMARY

Introduction- Develop multiple next-gen-based genomics analysis platforms; apply to genomic signatures of androgen receptor-driven progression to castration-recurrent prostate cancer (CR-CaP).

Key Research Accomplishments-

Progress- Multiple next-gen sequencing instruments have been obtained, subjected to quality assurance benchmark tests and put into use for research projects. No fewer than 17 RPCI researchers are involved in or completed next projects using the following techniques: whole genome sequencing/re-sequencing, transcriptome(RNA)-seq, exome-seq, ChIP-seq, miRNA-seq, methylome-seq and Faire-seq.

We continue to develop the bioinformatics screening platform to identify virus genomic sequences in human tumor samples using next-gen RNA-seq of randomly-primed libraries.

We have used LNCaP and PC3 cells expressing wt-, Y267F-, Y534F-AR +/- CA-Src or -Ack1 to develop AR-ChIP. We have developed or acquired human AS- and CR-CaP cell lines, xenograft tumors, mouse transgenic tumors and human cancer samples. RNA and DNA from these samples have been collected and tested for RIN values >7. In the case of frozen CaP tissues, tumor lesions have been first isolated by laser capture microscopy (LCM). To validate samples to be tested for RNA-seq, several sentinel AR-driven genes have been tested by qRT-PCR such as PSA and TMPRSS2. AR- and H3K4me3-ChIP-seq and RNA-seq have been completed on AS- and CR-CaP cell line and tumor sets, the data analyzed, primary Src-driven signatures of CR-CaP identified, and individual gene expression changes validated +/- AR-siRNA (to determine which are AR-dependent). These signatures are now being vetted against gene expression databases of human CR-CaP to validate as predictors of CR progression.

Reportable Outcomes-

23 manuscripts using the NGS capabilities funded by the current project have been published, including a report of whole bladder cancer genome sequencing by Carl Morrison, an analysis of a Vitamin D-regulated cistrome in prostate cancer progression by Moray Campbell, and a global analysis of FOXO1-regulated differential miRNA expression by Eugene Kandel.

Irwin Gelman's lab has developed human and mouse prostate cancer and tumor panels to identify androgen receptor-regulated genes controlled by Src and Ack1 tyrosine kinases in the context or progression to CR-CaP. These include congenic androgen-dependent and CR-CaP cancer cell lines pairs (e.g.- CWR22Pc and CWR22Rv1), as well as PC3 and LNCaP lines engineered to express either active Src or Ack1 in conjunction with WT, 267F or 534F alleles of the androgen receptor gene.

The Gelman Lab is also developing mouse transgenic models that cross a pro-metastatic phenotype (AKAP12-null) to several prostate cancer-prone phenotypes (prostate-specific Rb-, p53- or PTEN-null), so that the resulting tumors could be analyzed to identify the AR-cistrome involved in CR-CaP.

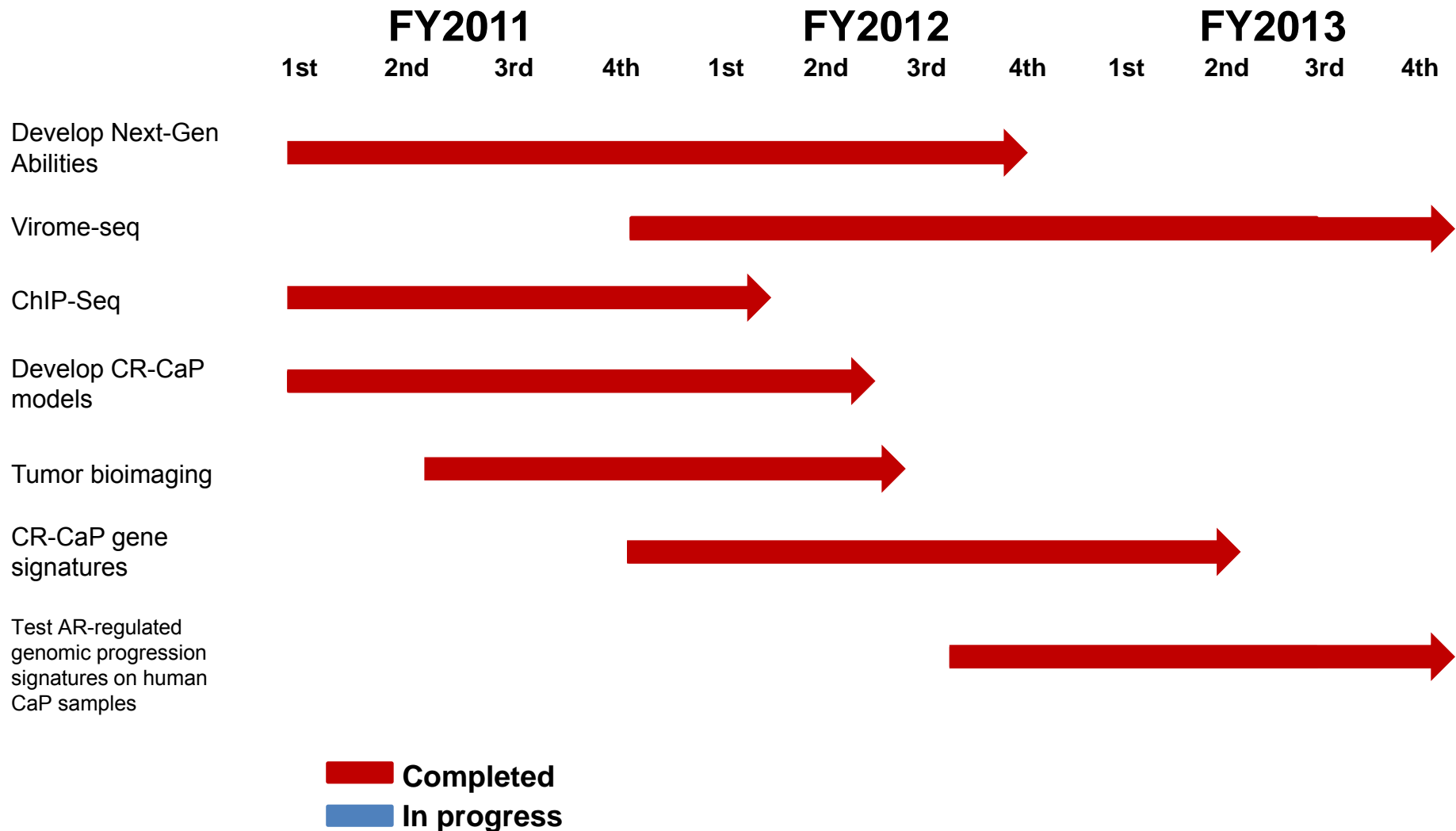
Conclusion- The funds provided to develop the Advanced Cancer Genomics Institute at RPCI have been used to successfully set up the appropriate next-gen instrumentation. This system has been reduced to practice using multiple next-gen technologies by multiple labs and groups at RPCI to address important cancer genomic questions and to lay the foundation for new cancer gene discoveries or new genetic prognostic/diagnostic markers for cancer progression. The research that will result from this Institute will greatly enhance our ability to provide personalized medical tests and treatments for cancer patients at RPCI and the U.S.

Advanced Cancer Genomics Institute:

Genetic Signatures and Therapeutic Targets in Cancer Progression

PI- Gelman, I.H., Ph.D.

W81XWH-11-2-0033



Introduction- Our aim is to develop multiple next-gen-based genomics analysis capabilities to be able to identify cancer-related signatures that predict progression to malignancy and that identify novel therapeutic pathways and targets. To this end, we are purchasing for the Advanced Cancer Genomics Institute within our Genomics Core resource Lab multiple next-gen instruments, including associated hardware and software platforms. Following the performance of quality-assurance testing, RPCI researchers will perform whole genome sequencing/re-sequencing, exome-seq, transcriptome-seq, miRNA-seq, ChIP-seq, microbiome-seq, virome-seq and copy number variation/loss of heterozygosity (CNV/LOH) analyses including the associated bioinformatics analyses. Based on the concentration of expertise at RPCI in prostate cancer pre-clinical and clinical models, most of the proposed work has been adapted to address genomic signatures of androgen receptor-driven progression to castration-recurrent prostate cancer (CR-CaP). The capabilities of this Core will foster our goal of developing practical approaches to personalized cancer medicine. The long-term aim is to develop full-operational next-gen expertise to facilitate the collaboration of RPCI researchers with other members of the National Functional Genomics Consortium.

Approach (from Statement of Work)-

- 1) Develop next-gen capabilities in the following platforms-
 - Transcriptome (RNA)-seq: differential gene expression
 - Exome-seq: exon usage variation
 - copy number variation/loss of heterozygosity (CNV/LOH)
 - ChIP-seq and FAIRE: transcription factor binding sites
 - microRNA-seq: miRNA expression profiles
 - microbiome: bacterial and viral sequence identification
- 2) Collect biomaterials from androgen-dependent (AD) and CR-CaP cell lines, xenograft tumors in mice, transgenic mice and humans.
- 3) Develop standard operating procedure (SOP) to isolate tumor tissue by laser capture microscopy (LCM), isolate and quality control (QC) DNA/RNA, link to outcome data.
- 4) Optimize bioinformatics:
 - marry and rectify genomics data from multiple platforms
 - correlate with outcome (e.g., CR-CaP progression)
 - develop preliminary progression signatures
 - test signatures on supervised data sets → optimize
 - long-term: signature correlation with unsupervised sets

Key Research Accomplishments-

The following report is divided by the experimental aims in the each of the Statement of Work sections below.

1. Develop the technical ability to identify copy number variations, gene and microRNA expression changes, microbiome diversity, and exon usage variation from cell lines and human cancer tissue DNA and RNA using next-gen sequencing platforms

Technical capabilities: The following next-gene analyses/projects have been successfully completed or started using our Core infrastructure, for which bioinformatics analyses by Dr. Song Liu have been completed or are currently ongoing:

Carl Morrison – Full genome resequencing and Exome Capture sequencing (**Fig. 1**)
 Gokul Das – transcriptome-seq
 Katerina Gurova – ChIP-seq
 Goodrich – ChIP-seq (**Fig. 2**)
 Campbell- ChIP-seq
 Gelman- ChIP-seq
 Pili – Faire-Seq

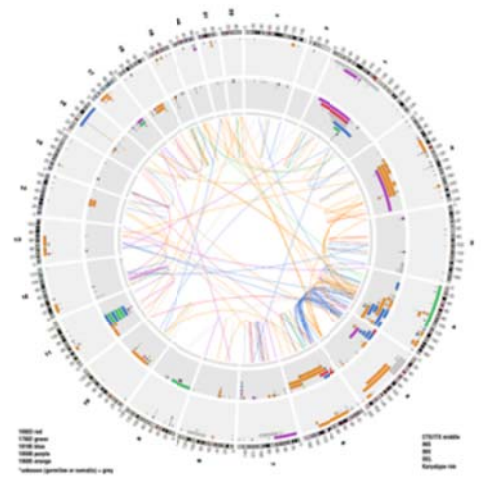


Figure 1. Bladder cancer mutation analysis of whole genome. Many unreported mutations, translocations and inversions were identified.

Bohua Hu – transcriptome-seq
 Gelman – transcriptome-seq
 Yuriy Ionov – shRNA library sequencing
 Eugene Kandel – miRNA-seq (**Fig. 3-4**)
 Higgins- methylome-seq
 Smiraglia/Gross- methylome-seq

Equipment: The following next-gen instruments were ordered and put into use during FY1 of the grant period:

- i) RainDance PCR-based gene analyzer, including a set of 3000 cancer gene-specific primer sets for tumor resequencing analysis.
- ii) Illumina miSeq including maintenance contract
- iii) Illumina iScan , slide scanner for next-gen sequencing data detection
- iv) Covaris E210: DNA shearer used to produce next-gen libraries
- v) Caliper Sciclone: liquid handling robotic station
- vi) Autoloader-2: robotic platform for high-throughput sequencing

The following examples are shown as to how our next-gen capabilities are generating usable data. Fig. 1 describes the mutations identified in human bladder cancers by genomic sequencing/resequencing performed by Carl Morrison. This analysis identifies at least two mutually exclusive mutation groups, KRas and FGFR7, as well as several interesting genetic translocation and transversions that might encode driver, oncogenic fusion proteins.

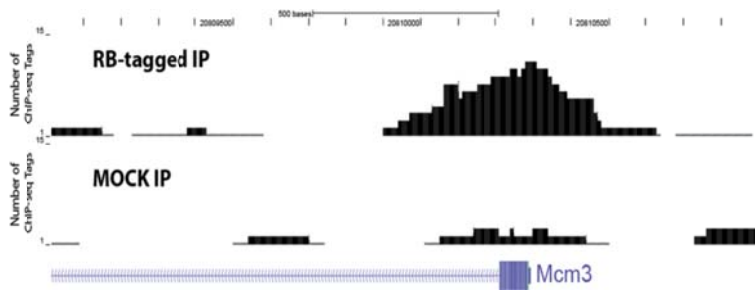


Figure 2. ChIP-seq analysis of the Mcm3 locus. Chromatin immunoprecipitated from tagged Rb1 MEFs or wild type MEFs (mock IP) using the FLAG antibody was analyzed by sequencing. The number of sequence tags mapping across the Mcm3 locus is shown. Note the enrichment in the tagged sample near the promoter of the gene.

Fig. 2 describes an Rb-ChIP-seq analysis over the Mcm3 gene locus in mouse embryo fibroblasts (MEF) by David Goodrich. This analysis shows novel Mcm3 promoter sites that bind Rb. Fig. 3-5 describe miRNA-seq analyses by Eugene Kandel

2. Develop the technical ability to identify novel virus family members from cell line and human cancer genetic material using custom Viro-Chips

Progress- We have produced transcriptome-seq from 12 next-gen libraries produced from laser capture microdissected Barrett's esophagus metaplasia biopsies (RNA RIN values >7), with another 33 RNA samples yet to be analyzed. Song Liu is developing bioinformatic masks against the NCBI virus genome database to identify potential viral co-factors using the PathSeq program (*Nature Biotechnol.*, 29393–396, 2011). These data can also be used to identify putative differential exon usage signatures of progression to esophageal high-grade dysplasia. Thus, rather than produce Viro-Chips, which requires the synthesis of >1300 oligonucleotides (a non-renewable resource), we have adopted the NGS approach followed by specific bioinformatics analyses.

3. Develop technical ability to identify gene sets regulated by specific transcription factors using ChIP-seq

Progress- In regards to the combined AR-ChIP-seq and transcriptome-seq data for castration-recurrent prostate cancer (CR-CaP), collaborative efforts from Drs. Gelman, Buck, Campbell, Kandel and Liu have begun to analyze Illumina libraries for androgen (DHT) responsive gene signatures from

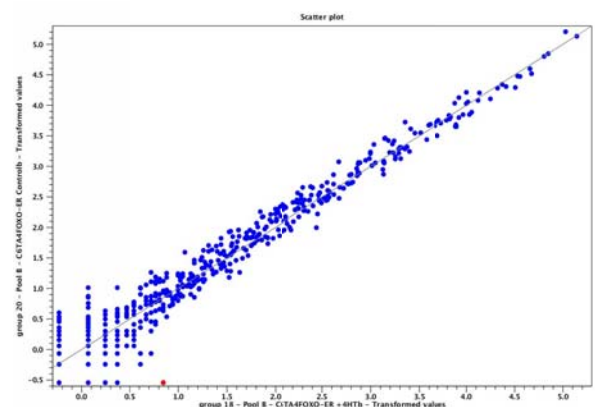


Figure 3. miRNA-seq analysis of FOXO-regulated miRNAs in HEK293 cells. Fluctuations of miRNAs after induction of FOXO transcriptional repressor showing downregulation of miR-506 (red dot).

LNCaP and PC-3 cell lines expressing ca-Src or –Ack1 tyrosine kinases +/- wt, Y267F or Y534F versions of the androgen receptor (AR)(Fig. 4), as well as from other androgen-dependent vs. CR-CaP cell line pairs (CWR22Pc vs. CWR22Rv1, CASP1-1 vs. CASP2-1, LNCaP vs. LNCaP-C4-2, VCaP vs. VCaP-CR) and tumor pairs (CWR22-AD vs. CWR22-CR, CASP1-1 vs. CASP2-1).

4. Develop transplantable castration-recurrent prostate cancer (CR-CaP) models of human and mouse prostate cancer lines in which androgen receptor activity is controlled by androgens or by modifications by Src Family or Ack1 tyrosine kinases.

Progress- We have developed or acquired multiple cell lines, xenograft tumors, transgenic tumors and human cancer samples that compare androgen-dependent (AD) to CR-CaP (see sect. 3 and Fig. 5). In addition, we have isolated human CWR22 tumors (AD or CR) grown in nude mice treated with Dasatinib or KXO1 (vs. vehicle). We have started to produce the following crosses that will produce further sets of AD vs. CR-CaP tumors for combined ChIP-seq/transcriptome-seq analyses: i) AKAP12-/-; Prostate-specific CRE-driven loss of PTEN, p53 or Rb, ii) prostate-specific PTEN loss; N-Myc^{hi}. We have also received permission from our GU-Disease Site Research Group, as well as from our internal Clinical Research Committee to obtain fresh human prostatectomy material to isolate RNA and DNA from AD- and CR-CaP cases. Roswell Park already has produced a 5-slide tumor microarray containing 722 CaP/matched normal biopsy samples for follow-up analyses. RNA and DNA from prostatectomy samples have been collected and tested for RIN values >7. In the case of frozen CaP tissues, tumor lesions have been first isolated by laser capture microscopy (LCM). To validate samples to be tested for RNA-seq, several sentinel AR-driven genes have been tested by qRT-PCR such as PSA and TMPRSS2. We are also receiving mouse C57Bl/6 transgenic prostate cancer cell lines, RM1 and RM9, from Tim Thompson (MD Anderson), which we will select for AD- and CR-growth *in vivo*, respectively.

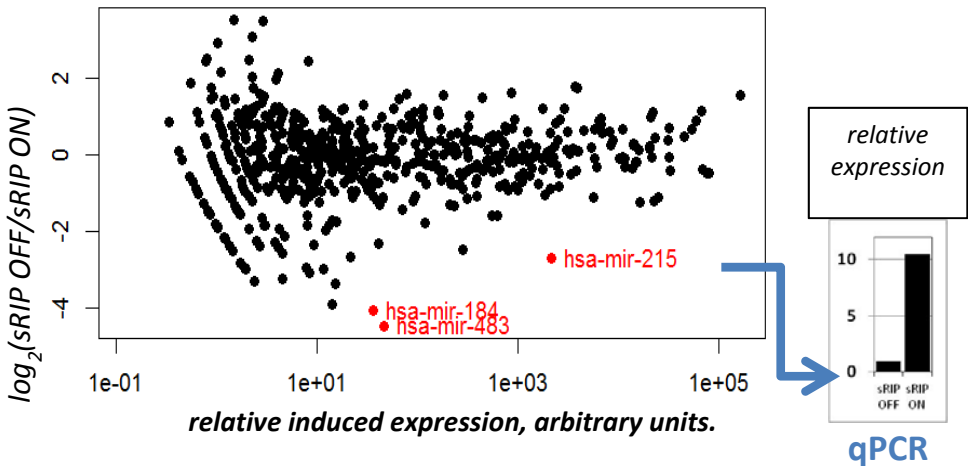


Figure 4. miRNAs induced by TNF ∇ . Next-gen sequencing we identified miRNAs that induced in HEK293 cells by TNF ∇ (not shown) and by elevated expression of short form of RIP1 (shown). Confirmatory experiments (qPCR) have been started on the commonly up-regulated miRNAs, with miR-215 shown.

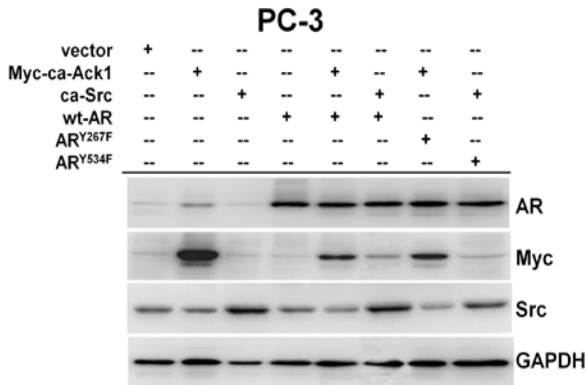


Figure 5. CR activation of AR by Src or Ack tyrosine kinase. Western blot analysis of AR (wt or Y→F mutants), Src, and Ack1 expression in PC-3 CaP cells. Src/Ack induce AR-mediated gene expression changes in the absence of androgens (DHT), and this is blocked by the co-expression of Y534F/Y267F AR mutants.

5. Develop non-invasive bioimaging techniques for marked primary and metastatic tumor cells in mice.
- Progress-** We are purchasing an IVIS Spectrum system from Caliper Sciences (now Perkin-Elmer). The involvement of our co-I, Mukund Seshadri, Ph.D. (Director, Bioimaging Core Lab), in this development prompted his inclusion for salary support. Dr. Seshadri is serving as the PI on an NIH Shared Instrumentation Grant to purchase a companion small animal MR instrument that will connect physically and functionally to the IVIS system that will allow integration of luminescence, X-Ray and MR data, all with capabilities for 3-D tomography of the integrated data. We have purchased several luciferase- and GFP-labeled cancer cell lines from Caliper (e.g.- B16F10, LLC, PC-3) that various researchers will use for mouse models of primary tumor and metastasis growth. Fig. 6 is an example in which human T47D breast cancer cells transduced with a

genomic shRNA library were imaged non-invasively 2 weeks after orthotopic injection of female SCID mice (Gelman Lab).

6. Derive gene expression signatures contributing to prostate cancer progression in mouse transgenic models, in human prostate cancer samples, and in mouse xenograft models of human prostate cancer.

Progress- bioinformatic analyses are in progress on the various next-gen data on CaP samples, which will be interrogated to identify potential signatures of CR-CaP progression. Confirmatory tests such as qRT-PCR and immunoblots have been performed to confirm gene expression changes. In addition, we have used a synthetic-lethal shRNA screen to confirm that expression of many of these genes is required for the selective proliferation of CR-CaP (as opposed to AS-CaP) cells. The first preliminary CR-CaP signature includes just under 20 genes that satisfy all these criteria. Importantly, AR expression- known to be upregulated in CR-CaP cells lines and in CR-CaP tumors, is one of the genes in this signature, confirming previous data in the literature that CR-CaP growth is dependent on AR but not on serum levels of androgens.

7. Test (supervised and unsupervised) AR-regulated genomic progression signatures on defined human CaP samples

Progress- In order to develop a CR-specific CaP signature driven by AR, we are comparing the gene expression (RNA-seq) vs. the AR-engaged genes (AR-ChIP-seq) in isogenic androgen-sensitive (AS) vs. CR-

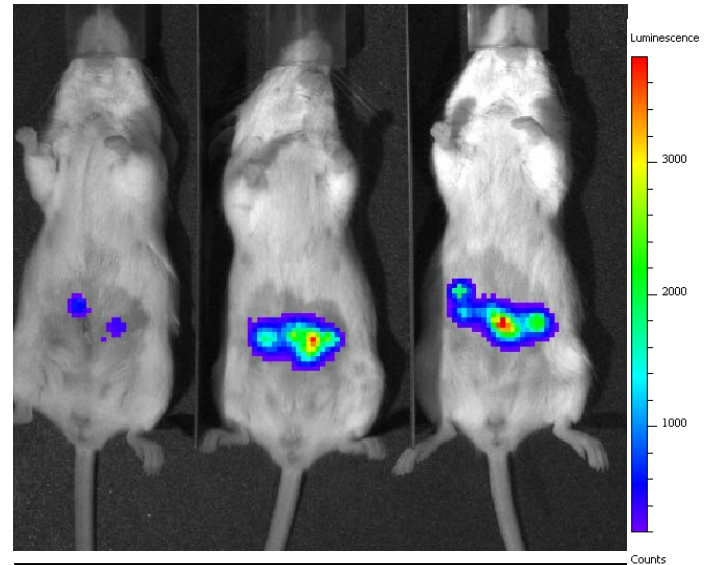


Figure 6. Example of IVIS imaging of orthotopic injection of T47D-luc2 cells, 2 weeks post-injection in mammary fat pads. Following IP injection of luciferin, anesthetized mice were subjected to Xenogen IVIS analysis of bioluminescence, the intensity of which is shown in the scale at right. The experiment is aimed at identifying genes that suppress spontaneous *in vivo* metastasis given the very low metastatic activity of the T47D line.

Samples	# reads per sample	Yield (million bases)	% of \geq Q30 Bases (PF)	Mean Quality Score (PF)
LN-CaP-Control	105456818	5378	98.32	38.8
LN-CaP + DHT	199430608	10171	94.62	37.17
V-CaP-Control	130188094	6640	97.08	38.27
V-CaP + DHT	110981604	5660	96.91	38.26
LN-CaP-SRC	196491943	10021	94.66	37.19
LN-CaP-SRC + DHT	198743718	10136	94.74	37.22

Table 1. RNA-seq analysis of Src-induced genes that contribute to CR-CaP growth. *in silico* potential CR gene progression signatures.

An example of RNA-seq and AR-ChIP-seq analyses that have been performed on LNCaP +/- DHT vs. LNCaP-Src +/- DHT is shown in Table 1. There was $>94\%$ data indicating better than 30-fold coverage of the transcriptome, which constitutes a very high standard for gene expression data depth. Moreover, there was no bias due to transcript length in our RNA-seq data sets (Fig. 7). Interestingly, although DHT treatment induced many up-regulated gene expression changes in LNCaP cells, Src activation of AR caused a preponderance of down-regulated gene expression changes (Fig. 8).

We have now begun to compare the Src- and Ack1-induced, AR-dependent genomic signature that drives CR-CaP growth *in vivo*, and have compared these with published AR cistrome data from clinical CR-

CaP samples (Sharma et al., *Cancer Cell*, 2013, 23:35-47). Table 2 shows a preliminary set of Src signature genes common to CR- but not to androgen-dependent CaP clinical samples. We are honing this signature, to increase statistical power, on the assumption that it could be used as a non-biased predictor of CR-CaP progression in early CaP cases.

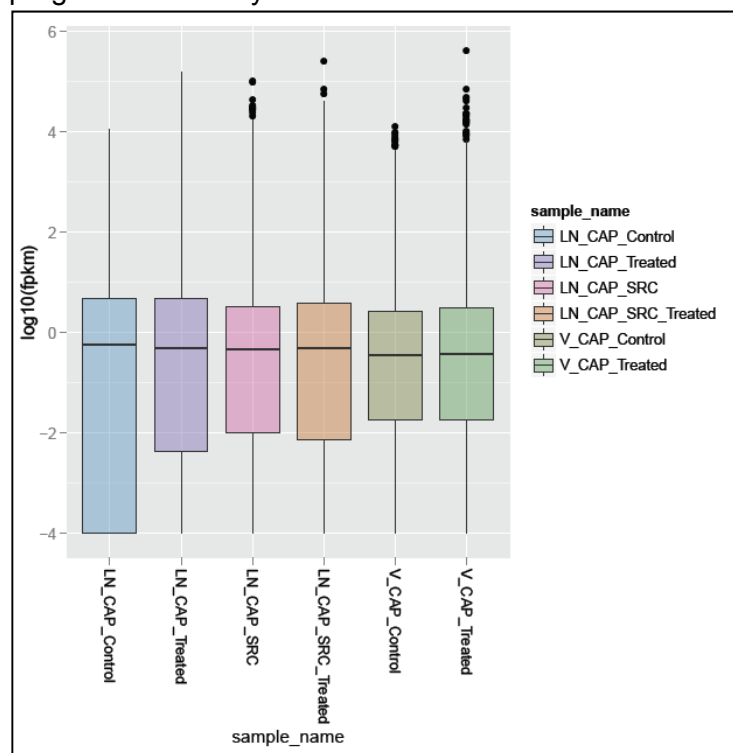


Figure 7. Normalization for transcript length vs. expression level. An algorithm for “Fragments per kilobase of transcript per million mapped reads” (FPKM; part of Cufflinks, UC-Berkeley; <http://cufflinks.cbc.umd.edu>) was applied to the RNA-seq data. Our analyses show that comparable mean values of FPKM, that is, no bias due to gene length.

DHT regulated genes overlapping AR targets in Human CRPC tissue*	Src regulated genes overlapping AR targets in Human CR-CaP tissue*
PDE3A	TM4SF1
MYBPC1	JAG1
NCAPD3	DPP4
STEAP4	ADAMTSL3
SPOCK1	PLEK2
RHOU	VAV3
NDRG1	C10orf81
THSD7A	F5
TG	NOV
TARP	FRY
KLK2	HMGCS2
IGF1R	LHX6
SDK1	TMC1

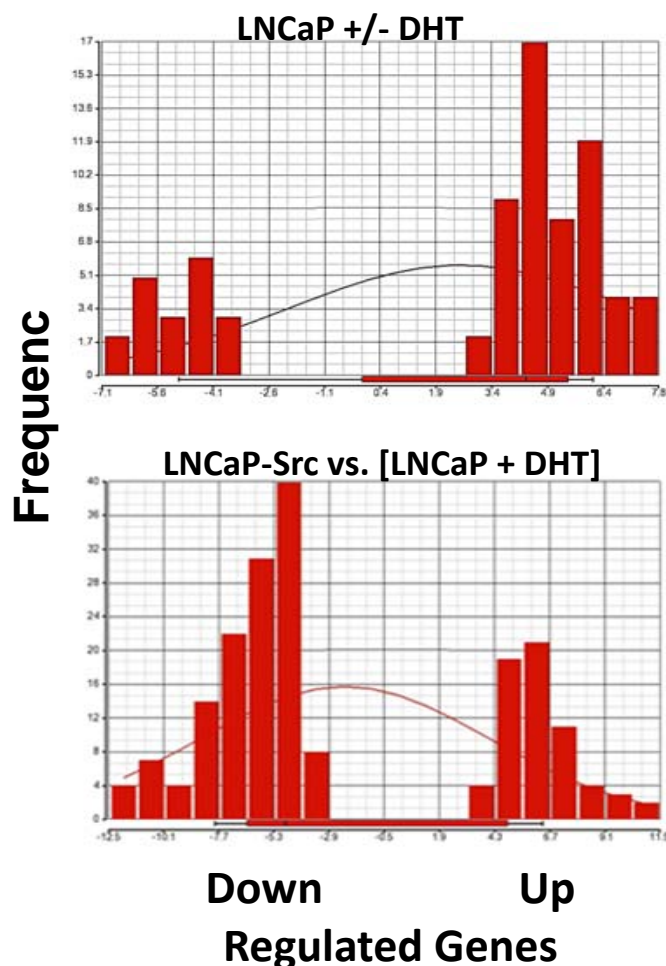


Figure 8. Fold-changes in up- and down-regulated gene expression changes in LNCaP cells +/- DHT or in LNCaP-Src cells vs. DHT-treated LNCaP cells.

Reportable Outcomes-

-Publications: the first set of manuscripts arising from the NGS capabilities funded through the NFGC/TATRC mechanism have been published:

- 1: Hennon MW, Yendamuri S. Advances in lung cancer surgery. *J Carcinog*.2012;11:21. doi: 10.4103/1477-3163.105341. Epub 2012 Dec 31. PubMed PMID:23346014.
- 2: Leonova KI, Brodsky L, Lipchick B, Pal M, Novototskaya L, Chenchik AA, Sen GC, Komarova EA, Gudkov AV. p53 cooperates with DNA methylation and a suicidal interferon response to maintain epigenetic silencing of repeats and noncoding RNAs. *Proc Natl Acad Sci U S A*. 2013 Jan 2;110(1):E89-98. doi:10.1073/pnas.1216922110. Epub 2012 Dec 10. PubMed PMID: 23236145; PubMed Central PMCID: PMC3538199.
- 3: Heller ER, Gor A, Wang D, Hu Q, Lucchese A, Kanduc D, Katdare M, Liu S, Sinha AA. Molecular signatures of basal cell carcinoma susceptibility and pathogenesis: A genomic approach. *Int J Oncol*. 2013 Feb;42(2):583-96. doi:10.3892/ijo.2012.1725. Epub 2012 Nov 30. PubMed PMID: 23229765.
- 4: Yan L, Ma C, Wang D, Hu Q, Qin M, Conroy JM, Sucheston LE, Ambrosone CB, Johnson CS, Wang J, Liu S. OSAT: a tool for sample-to-batch allocations in genomics experiments. *BMC Genomics*. 2012 Dec 10;13:689. doi:10.1186/1471-2164-13-689. PubMed PMID: 23228338.
- 5: Singh PK, Doig CL, Dhiman VK, Turner BM, Smiraglia DJ, Campbell MJ. Epigenetic distortion to VDR transcriptional regulation in prostate cancer cells. *J Steroid Biochem Mol Biol*. 2012 Oct 23. doi:pii: S0960-0760(12)00199-9. 10.1016/j.jsbmb.2012.10.002. [Epub ahead of print] PubMed PMID: 23098689.
- 6: Patnaik SK, Yendamuri S, Kannisto E, Kucharczuk JC, Singhal S, Vachani A. MicroRNA expression profiles of whole blood in lung adenocarcinoma. *PLoS One*. 2012;7(9):e46045. doi: 10.1371/journal.pone.0046045. Epub 2012 Sep 28. PubMed PMID: 23029380; PubMed Central PMCID: PMC3460960.
- 7: Campbell MJ, Turner BM. Altered histone modifications in cancer. *Adv Exp Med Biol*. 2013;754:81-107. doi: 10.1007/978-1-4419-9967-2_4. Review. PubMed PMID: 22956497.
- 8: Garcia H, Miecznikowski JC, Safina A, Commane M, Ruusulehto A, Kilpinen S, Leach RW, Attwood K, Li Y, Degan S, Omilian AR, Guryanova O, Papantonopoulou O, Wang J, Buck M, Liu S, Morrison C, Gurova KV. Facilitates chromatin transcription complex is an "accelerator" of tumor transformation and potential marker and target of aggressive cancers. *Cell Rep*. 2013 Jul 11;4(1):159-73. doi: 10.1016/j.celrep.2013.06.013. Epub 2013 Jul 3. PubMed PMID: 23831030.
- 9: Singhal R, Bard JE, Nowak NJ, Buck MJ, Kandel ES. FOXO1 regulates expression of a microRNA cluster on X chromosome. *Aging (Albany NY)*. 2013 May;5(5):347-56. PubMed PMID: 23748164; PubMed Central PMCID: PMC3701110.
- 10: Golubovskaya VM, Sumbler B, Ho B, Yemma M, Cance WG. MiR-138 and MiR-135 Directly Target Focal Adhesion Kinase, Inhibit Cell Invasion, and Increase Sensitivity to Chemotherapy in Cancer Cells. *Anticancer Agents Med Chem*. 2013 Feb 15. [Epub ahead of print] PubMed PMID: 23438844.
- Koochekpour S, Marlowe T, Singh KK, Attwood K, Chandra D. Reduced Mitochondrial DNA Content Associates with Poor Prognosis of Prostate Cancer in African American Men. *PLoS One*. 2013 Sep 23;8(9):e74688. PubMed PMID: 24086362; PubMed Central PMCID: PMC3781126.
- 11.: Yao S, Graham K, Shen J, Campbell LE, Singh P, Zirpoli G, Roberts M, Ciupak G, Davis W, Hwang H, Khoury T, Bovbjerg DH, Jandorf L, Pawlish KS, Bandera EV, Liu S, Ambrosone CB, Zhao H. Genetic variants in microRNAs and breast cancer risk in African American and European American women. *Breast Cancer Res Treat*. 2013 Sep 24. [Epub ahead of print] PubMed PMID: 24062209.
- 12: Yu X, Song H, Xia T, Han S, Xiao B, Luo L, Xi Y, Guo J. Growth inhibitory effects of three miR-129 family members on gastric cancer. *Gene*. 2013 Dec 10;532(1):87-93. doi: 10.1016/j.gene.2013.09.048. Epub 2013 Sep 20. PubMed PMID: 24055727.
- 13: Baysal BE, De Jong K, Liu B, Wang J, Patnaik SK, Wallace PK, Taggart RT. Hypoxia-inducible C-to-U coding RNA editing downregulates SDHB in monocytes. *PeerJ*. 2013 Sep 10;1:e152. doi: 10.7717/peerj.152. PubMed PMID: 24058882; PubMed Central PMCID: PMC3775634.
- 14: Luo W, Hu Q, Wang D, Deeb KK, Ma Y, Morrison CD, Liu S, Johnson CS, Trump DL. Isolation and genome-wide expression and methylation characterization of CD31+ cells from normal and malignant human prostate tissue. *Oncotarget*. 2013 Sep;4(9):1472-1483. PubMed PMID: 23978847.
- 15: James SR, Cedeno CD, Sharma A, Zhang W, Mohler JL, Odunsi K, Wilson EM, Karpf AR. DNA methylation and nucleosome occupancy regulate the cancer germline antigen gene MAGEA11. *Epigenetics*. 2013 Jul 9;8(8). [Epub ahead of print] PubMed PMID:23839233.

16: Verone AR, Duncan K, Godoy A, Yadav N, Bakin A, Koochekpour S, Jin JP, Heemers HV. Androgen-responsive serum response factor target genes regulate prostate cancer cell migration. *Carcinogenesis*. 2013 Aug;34(8):1737-46. doi: 10.1093/carcin/bgt126. Epub 2013 Apr 10. PubMed PMID: 23576568; PubMed Central PMCID: PMC3731805.

17: Heller ER, Gor A, Wang D, Hu Q, Lucchese A, Kanduc D, Katdare M, Liu S, Sinha AA. Molecular signatures of basal cell carcinoma susceptibility and pathogenesis: a genomic approach. *Int J Oncol*. 2013 Feb;42(2):583-96. doi:10.3892/ijo.2012.1725. Epub 2012 Nov 30. PubMed PMID: 23229765.

18: Morrison CD, Liu P, Woloszynska-Read A, Zhang J, Luo W, Qin M, Bshara W, Conroy JM, Sabatini L, Vedell P, Xiong D, Liu S, Wang J, Shen H, Li Y, Omilian AR, Hill A, Head K, Guru K, Kunnev D, Leach R, Eng KH, Darlak C, Hoeflich C, Veeranki S, Glenn S, You M, Pruitt SC, Johnson CS, Trump DL. Whole-genome sequencing identifies genomic heterogeneity at a nucleotide and chromosomal level in bladder cancer. *Proc Natl Acad Sci U S A*. 2014 Jan 27. [Epub ahead of print] PubMed PMID: 24469795.

19: Ambrosone CB, Young AC, Sucheston LE, Wang D, Yan L, Liu S, Tang L, Hu Q, Freudenheim JL, Shields PG, Morrison CD, Demissie K, Higgins MJ. Genome-wide methylation patterns provide insight into differences in breast tumor biology between American women of African and European ancestry. *Oncotarget*. 2013 Nov 29. [Epub ahead of print] PubMed PMID: 24368439.

20: Liu B, Morrison CD, Johnson CS, Trump DL, Qin M, Conroy JC, Wang J, Liu S. Computational methods for detecting copy number variations in cancer genome using next generation sequencing: principles and challenges. *Oncotarget*. 2013 Nov;4(11):1868-81. PubMed PMID: 24240121; PubMed Central PMCID: PMC3875755.

21: Yadav N, Chandra D. Mitochondrial DNA mutations and breast tumorigenesis. *Biochim Biophys Acta*. 2013 Dec;1836(2):336-44. doi: 10.1016/j.bbcan.2013.10.002. Epub 2013 Oct 16. Review. PubMed PMID: 24140413; PubMed Central PMCID: PMC3891589.

22: Baysal BE. Mitochondrial complex II and genomic imprinting in inheritance of paraganglioma tumors. *Biochim Biophys Acta*. 2013 May;1827(5):573-7. doi: 10.1016/j.bbabbio.2012.12.005. Epub 2013 Jan 2. Review. PubMed PMID: 23291190.

23: Yan L, Ma C, Wang D, Hu Q, Qin M, Conroy JM, Sucheston LE, Ambrosone CB, Johnson CS, Wang J, Liu S. OSAT: a tool for sample-to-batch allocations in genomics experiments. *BMC Genomics*. 2012 Dec 10;13:689. doi: 10.1186/1471-2164-13-689. PubMed PMID: 23228338; PubMed Central PMCID:PMC3548766.

-Song Liu, with Michael Higgins, Lara Sucheston, Christine Ambrosone, Candace Johnson and Dominic Smiraglia, have developed and published a novel bioinformatics program, IMA, to analyze methylome-seq data (*Bioinformatics* 2012). Song Liu also published a program, VPA, to handle next-gen sequencing variants based on user-specified frequency patterns (*BMC Res. Notes*, 2012).

-Irwin Gelman's lab has developed human and mouse prostate cancer and tumor panels to identify androgen receptor-regulated genes controlled by Src and Ack1 tyrosine kinases in the context or progression to CR-CaP. These include congenic androgen-dependent and CR-CaP cancer cell lines pairs (e.g.- CWR22Pc and CWR22Rv1), as well as PC3 and LNCaP lines engineered to express either active Src or Ack1 in conjunction with WT, 267F or 534F alleles of the androgen receptor gene.

-The Gelman Lab is developing mouse transgenic models that cross a pro-metastatic phenotype (AKAP12-null) to several prostate cancer-prone phenotypes (prostate-specific Rb-, p53- or PTEN-null), so that the resulting tumors could be analyzed to identify the AR-cistrome involved in CR-CaP.

-IVIS systems have been developed to monitor the effects of cancer genetics on tumor growth and metastasis *in vivo*. One example is to monitor primary LNCaP and T47D orthotopic tumor growth (prostate and mammary gland, respectively) and then to monitor the production of spontaneous metastases following the transduction of a genomic shRNA library. Another example is to test the metastasis of B16F10-luc2 mouse melanoma cells from orthotopic primary tumors grown in genetically susceptible transgenic and syngeneic mouse hosts.

Conclusion- The funds provided to develop the Advanced Cancer Genomics Institute at RPCI have been used to successfully set up the appropriate next-gen instrumentation. This system has been reduced to practice using multiple next-gen technologies by multiple labs and groups at RPCI to address important cancer genomic questions and to lay the foundation for new cancer gene discoveries or new genetic

prognostic/diagnostic markers for cancer progression. The research that will result from this Institute will greatly enhance our ability to provide personalized medical tests and treatments for cancer patients at RPCI and the U.S.